

REVIEW ARTICLE

# Modulation of inflammasome activity by *Porphyromonas gingivalis* in periodontitis and associated systemic diseases

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Inflammasomes are large multiprotein complexes localized in the cytoplasm of the cell. They are responsible for the maturation of pro-inflammatory cytokines such as interleukin-1 $\beta$  (IL-1 $\beta$ ) and IL-18 as well as for the activation of inflammatory cell death, the so-called pyroptosis. Inflammasomes assemble in response to cellular infection, cellular stress, or tissue damage; promote inflammatory responses and are of great importance in regulating the innate immune system in chronic inflammatory diseases such as periodontitis and several chronic systemic diseases. In addition to sensing cellular integrity, inflammasomes are involved in the homeostatic mutualism between the indigenous microbiota and the host. There are several types of inflammasomes of which NLRP3 is best characterized in microbial pathogenesis. Many opportunistic bacteria try to evade the innate immune system in order to survive in the host cells. One of these is the periodontopathogen *Porphyromonas gingivalis* which has been shown to have several mechanisms of modulating innate immunity by limiting the activation of the NLRP3 inflammasome. Among them, ATP/P2X<sub>7</sub>-signaling is recently associated not only with periodontitis but also with development of several systemic diseases. The present paper reviews multiple mechanisms through which *P. gingivalis* can modify innate immunity by affecting inflammasome activity.

**Keywords:** innate immunity; subversion; inflammasomes; inflammation; *Porphyromonas gingivalis*; persistence; periodontitis; systemic diseases

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The innate immune system is the first line of defense against microbial pathogens. It is initiated by genome-encoded pattern recognition receptors (PRRs) that respond to invading microorganisms. PRRs recognize microbial pathogen-associated molecular patterns (PAMPs). This leads to activation of host defense pathways to clear the infection (1). In addition to microbial components, the receptors can respond to danger-associated molecular patterns (DAMPs) derived from the host (ATP, DNA, cholesterol crystals). Also environmental irritants such as asbestos, silica, alum, and nanoparticles can stimulate inflammasome-driven inflammation (2, 3). In the detection of pathogens, toll-like receptors (TLRs) are of crucial importance. They recognize distinct PAMPs and participate in the first line of defense against invading pathogens, playing a significant role in inflammation and immune cell regulation. Other well characterized evolutionary conserved PRRs

involved in innate immune defense are retinoic acid-inducible gene-I (RIG-I) receptors, C-type lectin receptors (CLRs), and nucleotide-binding domain (NOD)-like receptors (4, 5). All these receptors are expressed by several cell types such as macrophages, neutrophils, monocytes, and epithelial cells (6).

The activation of PRRs and their post-receptor signaling can stimulate recruitment of the so-called inflammasome complexes (1, 3, 6–15). Inflammasome is a relatively new concept introduced by Tschopp et al. in 2002 (7). Later, Tschopp also introduced the concept of metabolic syndrome that senses metabolic stress and contributes to the metabolic syndrome associated with obesity and type 2 diabetes (16).

The aims of the present paper are to give a brief description of inflammasome compositions and functions and to systematically review how *Porphyromonas gingivalis*, a putative keystone pathogen in chronic periodontitis (17),

well known for its way to manipulate the innate immune system (18), may carry out/modulate inflammasome activation in chronic periodontitis and certain other chronic systemic infections. The role of inflammasome and danger molecule signaling in the oral cavity was recently reviewed by Yilmaz and Lee (6).

### Inflammasomes

Inflammasomes are multiprotein complexes localized within the cytoplasm of the cell. They are engaged in the maturation of pro-inflammatory cytokines such as interleukin-1 $\beta$  (IL-1 $\beta$ ) and interleukin-18 (IL-18) (19). After infection or cellular stress, inflammasomes are assembled, activated, and involved in host defense and in the pathophysiology of diseases (20). Inflammasomes follow canonical or non-canonical pathways. A typical functional canonical inflammasome complex consists of a nucleotide-binding leucine-rich repeat (NLR) protein, an adaptor molecule apoptosis-associated speck-like protein containing a caspase activation and recruitment domain (ASC) and procaspase-1 (21). A typical non-canonical inflammasome is the one activating caspase-11, which so far is an understudied pro-inflammatory caspase (22).

A unique scaffolding protein (NLR) dictates the formation of inflammasomes. Mutations in members of the NLR family have been linked to various inflammatory diseases consistent with the fact that these molecules play an important role in host–pathogen interactions and the inflammatory response (23). Each inflammasome contains a unique sensor protein of the NLR superfamily or the PYRIN and HIN-200 domain-containing (PYHIN) superfamily (10). In NLRs, signaling is exerted by caspase activation and the so-called caspase activation and recruitment domains (CARDs). These can recruit caspase-1 directly or by PYRIN domains recruiting caspase-1 *via* the CARD-PYRIN-containing adaptor protein ASC (10). The adaptor protein mediates a critical step in innate immune signaling by bridging the interaction between the pathogen recognition receptors and caspase-1 in inflammasome complexes (24).

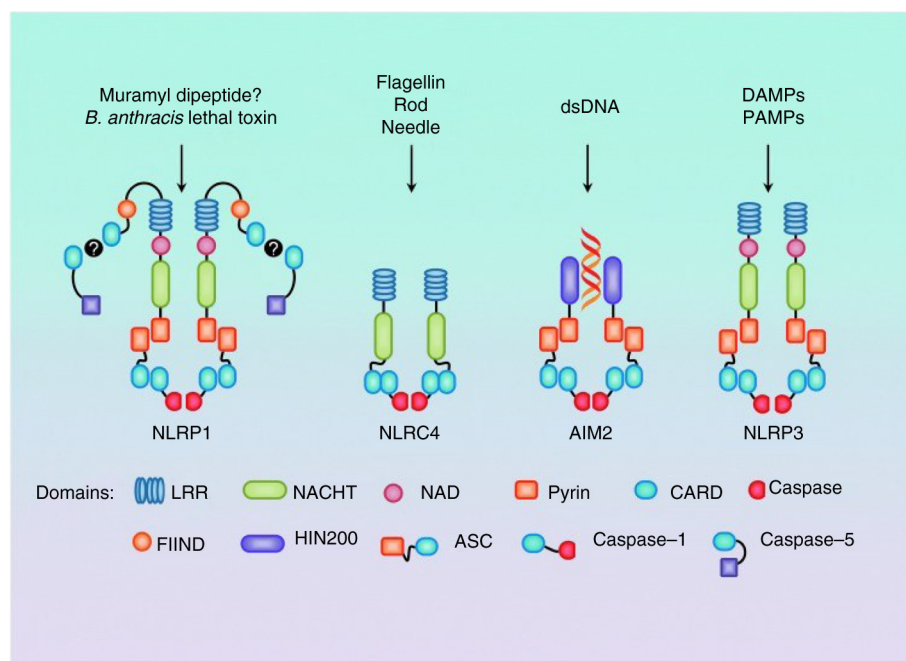
Inflammasomes have a critical role in initiating innate immune responses, particularly by acting as platforms for activation of the inflammatory caspase proteases. Among these, caspase-1 initiates innate immune responses by specifically cleaving of pro-IL-1 $\beta$  and pro-IL-18 and mediates their maturation and release (10). These cytokines promote recruitment of phagocytes, angiogenesis, epithelial cell repair, and regulation of cytokines and chemokine production by other immune cells at the site of infection or injury (reviewed by Hao et al. (25)). Inflammasomes also take part in the host defense independent of their classic cytokine targets IL-1 $\beta$  and IL-18.

Caspase-1 and 11 can start a rapid and inflammatory form of cell death, the so-called pyroptosis. They are distinct from caspases classically involved in apoptosis. Pyroptosis, a program of cellular self-destruction that is intrinsically inflammatory, results from osmotic pressure created by caspase-1-dependent formation of membrane pores (26, 27) and is associated with rapid release of cytosolic contents. This process can restrict intracellular replication of invasive bacterial pathogens (28) and probably acts in synergy with the recruitment of neutrophils by IL-1 $\beta$  to restrict replication of bacteria *in vivo* (10).

Inflammasomes sense cellular integrity and tissue health. When cell homeostasis is disrupted, inflammation is caused by the release of cytokines. A large amount of infectious and noxious insults can assemble these special structures. Thus inflammasomes may have a role in bacterial, parasitic, fungal, and viral infections (29). Inflammasomes also sense products and endogenous signals that indicate loss of cellular homeostasis (10, 15) and can be active both in periodontitis and several systemic diseases (6, 30).

Several distinct inflammasomes have currently been described, each of which is activated by unique stimuli (Fig. 1). Inflammasomes such as NLRP1 (nucleotide-binding domain-like receptor protein) inflammasome1, NLRP3, NLRP4, NLRP6, NLRP/NLRP12, and AIM2 (Absent In Melanoma 2) have been recognized acting in the host defense against intracellularly invading pathogens. Some inflammasomes are particularly well characterized for their role in bacterial recognition such as NLRC4, NLRP3, and AIM2 (Fig. 1). Also there may be more paths to IL-1 $\beta$  and IL-18 generation than *via* caspase-1 (1). It is likely that other caspase-1-dependent effector cytokines are produced by other proteases during infection (31). Several pathogens have been found to develop strategies to counteract inflammasomes (the so-called pathogenic stealth mechanisms) (32). Thus, *Staphylococcus aureus* can modify its cell wall peptidoglycan to prevent degradation by lysozymes through peptidoglycan O-acetyl transferase A which also strongly suppresses inflammasome activation and inflammation *in vitro* and *in vivo* (33).

While inflammasome activation is important to host defense, excessive inflammasome activation can be detrimental to health. Inflammasome hyperactivation is recently proposed to be the basis for autoinflammatory disease pathogenesis, whereas inflammasome regulated activity is central for appropriate host defense and protection from sepsis (16). Accordingly, there is need for a balance between resolution of infection and excessive inflammation (1). It should also be kept in mind that several pathogenic bacteria, for example, *Yersinia pestis*, *Salmonella*, and *Listeria monocytogenes* activate multiple inflammasomes demonstrating redundancy of



**Fig. 1.** Major inflammasomes with known stimulators. In NLRP1 muramyl dipeptide and *Bacillus anthracis* lethal toxin can directly cause caspase-5 processing. NLRC4 activation is mostly related to components of Gram-negative bacteria. In AIM2, double-stranded DNA (dsDNA) binds to the HIN200 domain and requires ASC for processing of caspase-1. Also, NLRP3 requires ASC and caspase-1. It is activated in response to both exogenous and endogenous danger signals. (From ref. 19 with permission.)

inflammatory receptors *in vitro* and *in vivo* (34, 35). It is also possible that several NLRs, AIM2s, and caspases co-operate during infections (1), which may be necessary for optimal responses to be obtained.

In inflammasome-mediated cytokine release, a multi-step activation pathway is followed. First, there is an NF- $\kappa$ B-dependent upregulation of the inactive pro-forms of IL-1 $\beta$  and IL-18 and of some NLRs like NLRP3; thereafter, activation of the NLR or AIM2, and inflammasome formation occurs (1). Some cells have simpler activation pathways because they have higher basal levels of the pro-forms of IL-1 $\beta$  and IL-18.

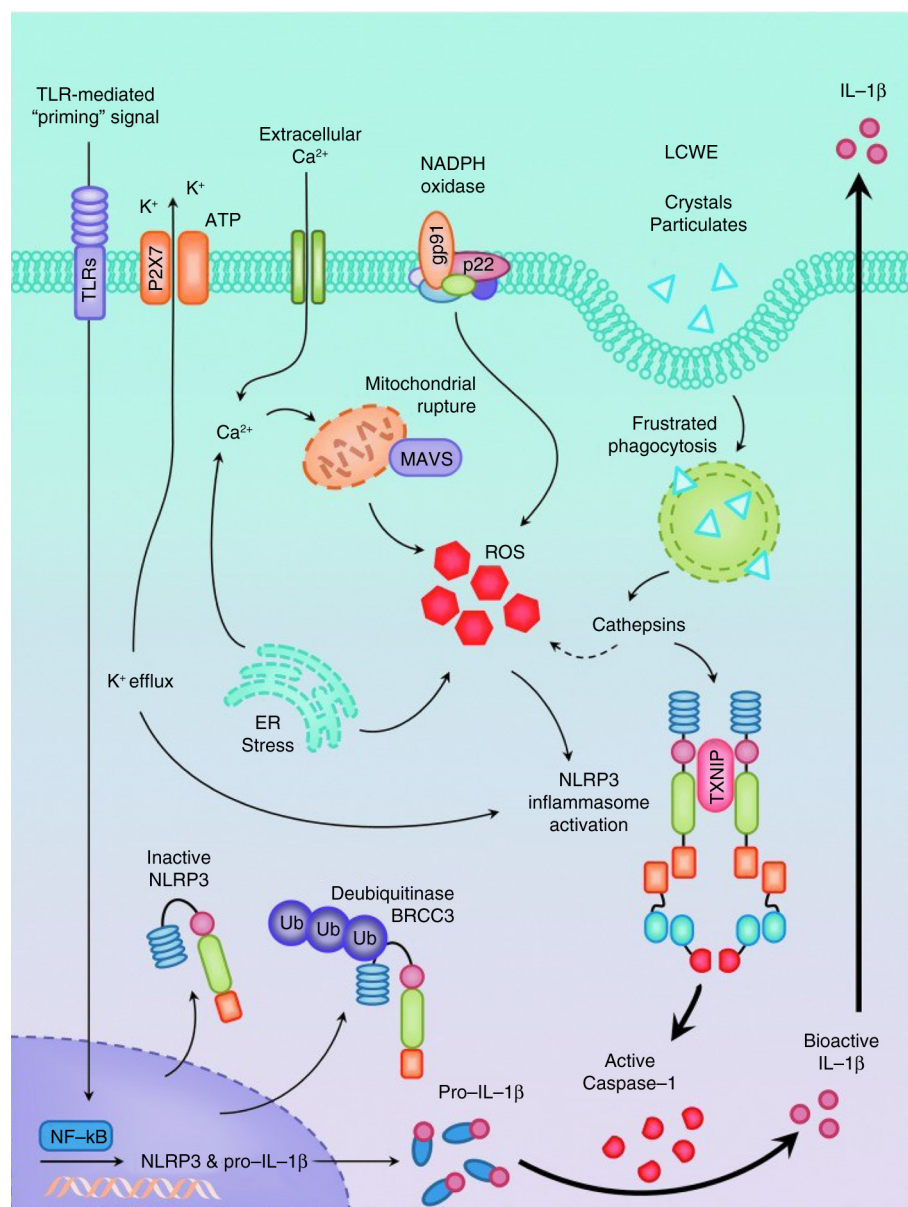
Interestingly, inflammasomes are also involved in the homeostatic mutualism between host and commensals. In the intestine, one inflammasome function is to control the composition of the microbiota (36). The NLCP4 inflammasome, expressed by intestinal phagocytes in particular, plays a major role to discriminate between commensal and pathogenic microbes and initiate a harmful response to the latter (37). Thus, inflammasomes may have a variety of roles regulating homeostasis in the intestinal tract and microbial ecology preventing the emergence of pathobionts (25, 38). Inflammasome-deficient mice exhibited an aberrant microbial community that triggered an enhanced inflammatory reaction in the intestine (39). This microbial dysbiosis affected the physiology and pathophysiology both locally in the intestine and sys-

temically and might contribute to the pathogenesis of intestinal bowel disease (10).

### The NLRP3 inflammasome

NLRP3 is part of one of the best-studied inflammasome complexes. It consists of the NLRP3 scaffold, the ASC adaptor, and procaspase-1 (3). Two steps are required to activate the NLRP3 inflammasome (25). The first step is initiated by microbial ligands or endogenous cytokines and is needed to induce upregulation of NLRP3 protein expression (2, 40). NF- $\kappa$ B activation and reactive oxygen species (ROS) are required for this step. The second step is activation of NLRP3 by microbial stimuli or endogenous molecules (25). NLRP3 is activated by several microbial-derived ligands, including toxins (20, 37). The endogenous signals triggering NLRP3 activation include the danger signal ATP, fatty acids, particulate matter, necrosis, and necroptosis (reviewed by Hao et al. (25)). Also K<sup>+</sup> efflux, lysosome function, endoplasmic reticulum (ER) stress, intracellular calcium, ubiquitination, microRNAs, and particularly ROS have been proposed (reviewed by Abais et al. (19)) (Fig. 2). ROS may serve a 'kindling' or triggering factor for activation of the NLRP3 inflammasome as well as 'bonfire' or 'effector' molecules leading to pathological processes (19).

In monocytes and dendritic cells, TLR stimulation is adequate to induce caspase-1 activation and IL-1 $\beta$  production but not in macrophages (reviewed by Hao et al. (25)).



**Fig. 2.** Activation of the NLRP3 inflammasome as a two-step mechanism. Primary signals come from activation of toll-like receptors (TLRs) which are responsible for the upregulation of NLRP3 and pro-interleukin-1 $\beta$  (IL-1 $\beta$ ) in an NF- $\kappa$ B-dependent manner. Secondary signals come from several pathways: K<sup>+</sup> efflux via P2X<sub>7</sub> receptor activation via ATPe coupling, endoplasmic reticulum (ER) stress, mitochondrial dysfunction, NADPH oxidase, frustrated phagocytosis, and lysosomal rupture pathways. All these primary and secondary signals converge in the production of reactive oxygen species (ROS). (From ref. 19 with permission.)

In human monocytes, TLR stimulation promotes extracellular release of ATP which in turn stimulates the purinergic receptor P2X<sub>7</sub> needed for activation of the NLRP3 inflammasome. In dendritic cells, however, NLRP3 activation is independent of P2X<sub>7</sub> (41). This implies that in some cell types TLR can activate the NLRP3 inflammasome independent of extracellular mediators such as ATP. All NLRP3s have a unique sensor protein of the NLR or the PYHIN superfamily. These proteins seem to possess numerous mechanisms for sensing bacteria and initiating immune mechanisms (10).

#### The AIM2 inflammasome

AIM2 is a cytosolic binding receptor for double-stranded DNA (1). It is known to form an inflammasome and activate caspase-1 when bacteria and viruses are present (42–44). AIM2 consists of an N-terminal pyrin domain and a C-terminal DNA-binding HIN200 domain. It is the only known HIN200 domain-containing protein with capacity to mature IL-1 $\beta$  and IL-18 through interactions with ASC and caspase-1 (42). The AIM2 inflammasome is particularly important in the defense against intracellular bacterial and viral pathogens (43, 44).



## *P. gingivalis*

### Synergy

Several opportunistic pathogens have been shown to develop different mechanisms to inhibit inflammasome activation and function. Similarly, *P. gingivalis*, a proposed keystone organism in chronic periodontitis (17), has been found to manipulate innate immunity via a number of mechanisms (18). This bacterium has been postulated to suppress inflammasome activation as a mechanism for its low immunostimulatory activity and pathogenic synergy with other periodontal bacteria that are shown to be more immunogenic (45). *P. gingivalis* can also suppress inflammasome activation by *Fusobacterium nucleatum* and this may be a contribution from *P. gingivalis* to the synergy between the two periodontal bacteria (46–49). This specific inhibition appears to affect IL-1 $\beta$  and IL-18 processing and cell death in macrophages of both man and mouse. While *F. nucleatum* activated IL-1 $\beta$  processing through the NLRP3 inflammasome, *P. gingivalis*-mediated repression was not related to lowered levels of inflammasome components (45). *P. gingivalis* infection also influences endocytosis by preferentially suppressing endocytic pathways toward inflammasome activation. This represents a new mechanism of pathogen-mediated inflammasome inhibition (45). It should also be noted that although *P. gingivalis* inhibits an activation pathway that can kill the microorganism; this may not be the integral part of a general immune suppression strategy as *P. gingivalis* harnesses acute sustained inflammation that is relatively harmless to the bacterium. Indeed, periodontitis-associated bacteria could benefit from a nutritionally favorable inflammatory environment created by *P. gingivalis* (50).

### Nucleoside-diphosphate kinase

*P. gingivalis* uses its extracellularly secreted nucleoside-diphosphate kinase homologue (NDK) (51) to inhibit innate immune responses due to stimulation by extracellular ATP (ATPe) (52). ATPe acts as a danger signal that can alert the immune system about a present infection. ATPe binds to P2X<sub>7</sub> receptors (see below) and activates an inflammasome and caspase-1. Infection of gingival epithelial cells (GECs) resulted in inhibition of ATP-induced caspase-1 activation (52). *ndk*-deficient *P. gingivalis* was less effective in limiting ATP-mediated caspase-1 activation and secretion of IL-1 $\beta$  from infected cells. NDK therefore seems to play an important role in inhibiting P2X<sub>7</sub>-dependent inflammasome activation. The consequent inhibition of P2X<sub>7</sub>-mediated apoptosis and extension of the viability of GECs could make *P. gingivalis* survive for extended periods of time in the gingival epithelium and contribute to disease when other host and bacterial factors participate in tissue destruction. NDK

also reduced ATPe-mediated plasma membrane permeabilization of host cells in a dose-dependent manner (53).

In GECs, NDK of *P. gingivalis* promoted intracellular persistence by inhibiting ATP-induced ROS via P2X<sub>7</sub> receptor/NADPH oxidase signaling (54). This implied that GECs produced significant amounts of ROS in response to ATPe and that this depended on P2X<sub>7</sub>-signaling coupled with membrane-bound NADP oxidase and the mitochondrial respiratory chain. This novel signaling cascade may contribute to successful tissue persistence of this major pathogen.

Also, the secreted multi-functional effector molecule, NDK from *P. gingivalis* attenuated release of the high-mobility group protein B1 (HMGB1) (52). HMGB1 is a pro-inflammatory danger signal associated with chromatin in healthy cells. Lack of NDK reduced significantly the inhibition of ATP-dependent inflammasome activation and the release of pro-inflammatory cytokines in GECs (52). The findings suggested that NDK could play a significant role in the inhibition of P2X<sub>7</sub>-dependent inflammasome activation and HMGB1-release from infected GECs.

### The P2X<sub>7</sub> and P2X<sub>4</sub> receptors

The P2X<sub>7</sub> purinergic cell surface receptor, which is expressed on a variety of immune cells, including macrophages, functions as a second signal for assembly of the NLRP3 inflammasome (55). Purinergic signaling is essential for the release of IL-1 $\beta$  from cells infected with *P. gingivalis* (56). In macrophages P2X<sub>7</sub> has a dual role, as it was critical not only for ATPe-induced IL-1 $\beta$  secretion *in vitro* but also for intracellular pro-IL-1 $\beta$  processing (57). These findings also applied to the *in vivo* situation since the P2X<sub>7</sub> receptor expression was upregulated in a *P. gingivalis* oral infection model. Further, the P2X<sub>7</sub> receptor and NLRP3 transcription were found to be modulated in human chronic periodontitis (57), suggesting that the P2X<sub>7</sub> receptor also has a role in periodontal immunopathogenesis. The ability of *P. gingivalis* to modulate ATP/P2X<sub>7</sub>-signaling, to secrete NDK during infection in primary GECs, and its expression of other virulence factors, for example, gingipains and fimbriae, and promotion of peripheral artery disease (PAD) may link this bacterium to periodontitis and other systemic diseases such as rheumatoid arthritis, diabetes, obesity, multiple sclerosis, and pancreatic and kidney diseases (58, 59). Interestingly, gingipains may also affect inflammasome activation. Jung et al. (60) recently showed that the simultaneous protease action of Kgp and Rgps attenuates the caspase-1 activating potential of *P. gingivalis* in macrophages.

It is well known that infection of GECs with *P. gingivalis* requires an exogenous danger signal such as ATPe for activation of an inflammasome and caspase-1. This again will induce secretion of IL-1 $\beta$ . Generation

of ROS is also stimulated by ATPe. However, the mechanism of ROS generation and the role of purinergic receptors in inflammasome activation were not very clear until Hung et al. (61) demonstrated that the purinergic receptor P2X<sub>4</sub> is assembled with P2X<sub>7</sub> and its associated pore, pannexin-1. ROS production was induced by ATPe through a complex containing P2X<sub>4</sub>, P2X<sub>7</sub>, and pannexin-1. The P2X<sub>7</sub>-mediated ROS production can activate the NLRP3 inflammasome and caspase-1. Activation by P2X<sub>4</sub> alone induced generation of ROS but not inflammasome activation. Depletion or inhibition of P2X<sub>4</sub>, P2X<sub>7</sub>, or the pannexin complex markedly blocked IL-1 $\beta$  secretion in *P. gingivalis*-infected GECs after ATPe treatment (61). Accordingly, ROS is produced by stimulation of the P2X<sub>4</sub>/P2X<sub>7</sub>/pannexin-1 complex. This also means that P2X<sub>4</sub> acts as a positive regulator of inflammasome activation during infection with *P. gingivalis*.

### A2a adenosine receptor

Danger signals (DS) are molecules like adenosine that exert extracellular signaling derived from autocrine and/or paracrine secretions during inflammation and chronic diseases. Adenosine, which is a metabolite of ATP, has so far been little appreciated as a component of the innate immune system (62). ATPe is produced through a number of enzymatic reactions in normal, stressed, or infected tissues (63). It has recently been shown that *P. gingivalis* can use A2A adenosine receptor-coupled DS adenosine signaling as a means to proliferate and survive in primary GECs, possibly by down-regulating the pro-inflammatory response (62). *P. gingivalis* reduces extracellular nucleotide concentrations of ATP and thereby acts as a generator of adenosine which stimulates the bacterium's growth in primary GECs. This may be another anti-inflammatory immune response exerted by *P. gingivalis* to promote its survival in the oral mucosa.

### Phosphatidylserine

During *Chlamydia* infection of human epithelial, endothelial, granulocyte, or monocytic cells, phosphatidylserine (PS) is translocated from the inner to the outer leaflet and becomes exposed to the external side of the cell (64). PS exposure is an early marker of apoptosis associated with pro-inflammatory triggering of complement activation (65, 66). Yilmaz et al. (67) found that *P. gingivalis* after establishing itself in the nutritionally rich cytosol of the host cell can protect the infected cell from the host immune defense by reducing the inflammatory response after inducing transient externalization of PS. This would allow multiplication of *P. gingivalis* inside cells while protecting them from cytotoxic reactions of the immune system. It was also suggested that the bacterium blocks mitochondrion-dependent apoptosis to upkeep its intracellular lifestyle. This may permit successful spreading of *P. gingivalis* to adjacent and deeper host tissues.

### Inflammasome activity in *P. gingivalis*-induced periodontal disease and defense

GECs are important parts of the immune response to periodontal bacteria. They express a functional NLRP3 inflammasome (68). Much higher levels of inflammasome components were found in the gingival tissues from patients with chronic periodontitis than from healthy controls (14). It therefore seems reasonable to consider the inflammasome as an operational part of innate immunity against periodontitis.

While supragingival biofilm, causing gingivitis, increased the expression of caspase-1, ASC, AIM2, IL-1 $\beta$ , and IL-18 in gingival fibroblasts, subgingival biofilm, promoting periodontitis, enhanced caspase-1, ASC, AIM2, IL-1 $\beta$ , and IL-18 gene expression at lower concentrations, followed by their downregulation at higher concentrations (69). The authors proposed that high concentrations of bacterial virulence factors in sites with affluent immune mechanisms such as the biofilm-tissue interface can down-regulate host defense barriers, while the lower bacterial concentrations deeper in periodontal tissues can have a stimulatory effect on inflammatory responses.

When using a 10-species subgingival biofilm with *P. gingivalis* present, Belibasakis et al. (70) found a reduction in NLRP3 and IL-1 $\beta$  expression in human gingival fibroblasts after challenge for 6 h. The AIM2 expression was not affected. After exclusion of *P. gingivalis* from the biofilm, a partial rescue of NLRP3 and IL-1 $\beta$ -expression occurred. It was suggested that subgingival biofilms down-regulate NLRP3 and IL-1 $\beta$  expression partly due to *P. gingivalis* and that this dampening of the host innate immune responses may favor persistence and survival of biofilm species in periodontal tissues.

It has been recently shown that fimbriae of *P. gingivalis* inhibit ATPe-induced IL-1 $\beta$  secretion through the P2X<sub>7</sub> receptor in macrophages (56). Ramos-Junior et al. (57), however, found that NLRP3 is necessary for ATPe-induced IL-1 $\beta$  secretion as well as for caspase-1 activation irrespective of *P. gingivalis* fimbriae. Although IL-1 $\beta$  secretion from *P. gingivalis*-infected macrophages depended on NLRP3, its adaptor protein ASC, or caspase-1, the cleavage of intracellular IL-1 $\beta$  to the mature form occurred independently of NLRP3, its adaptor protein ASC, or caspase-1.

*P. gingivalis* dampened ATPe-induced IL-1 $\beta$  secretion in macrophages by means of its fimbriae in a purinergic P2X<sub>7</sub> receptor-dependent manner (56). In this study, the immune subversion of *P. gingivalis* was connected with the ability of fimbriae to reduce ATPe-induced macrophage secretion of IL-1 $\beta$  via P2X<sub>7</sub> activation. The authors held that this could be another molecular action of subversion of the immune system by *P. gingivalis*.

In THP-1 (leukemic monocytic) cells, *P. gingivalis*-induced IL-1 $\beta$  secretion and inflammatory cell death

through activation of caspase-1 (14). IL-1 $\beta$  secretion and pyroptotic cell death required both NLRP3 and AIM2 inflammasome activation *via* TLR2 and TLR4 signaling. The activation of the former was mediated by ATP release, the P2X<sub>7</sub> receptor, and lysosomal damage (14). These authors also suggested that *P. gingivalis*-induced NLRP3 activation depends on ATP release, K<sup>+</sup> efflux, and cathepsin B.

#### Underacylated lipopolysaccharide

*P. gingivalis* can use lipid A phosphatase to alter the lipid A composition of its lipopolysaccharide (LPS). This organism may therefore modulate the immune response by expressing an underacylated LPS (71–73). This LPS could bind but not activate caspase-11 (human caspase-4,5) which results in host cell lysis and reduces survival of bacteria (72, 74). Caspase-4 can be of specific importance to mucosal immunity since caspase-4 expression level and ability to become activated as a response to infection differ markedly from that of caspase-5. Caspase-4 has been suggested to provide key non-redundant discernment into rapid sensing and clearance of bacteria at mucosal tissues (21).

#### Atherosclerosis

There is mounting evidence that *P. gingivalis* can invade cardiovascular cells and tissues causing inflammation (75). The NLRP3 inflammasome has been suggested to have an important role in developing vascular inflammation and atherosclerosis (76, 77). In hyperlipidemic animals, *P. gingivalis* accelerated atherosclerosis (78). Wild-type challenge of apolipoprotein E-deficient, spontaneously hyperlipidemic (ApoE<sup>shl</sup>) mice with *P. gingivalis* increased IL-1 $\beta$ , IL-18, and TNF- $\alpha$  production in peritoneal macrophages and gingival or aortic gene expression of the NOD-like receptor family, NLRP3, IL-1 $\beta$ , pro-IL-18 and pro-caspase-1 (78). Fimbriae were found to bring increased tissue invasiveness and pro-inflammatory ability to *P. gingivalis*. It was also demonstrated that *P. gingivalis* activates innate immune cells through the NLRP3 inflammasome compared with a KDP136 (gingipain-null) or a KDP150 (FimA-deficient) mutant.

Inflammation-related induction of AIM2 in vascular cells and atherosclerotic lesions has suggested a role for AIM2 in vascular pathogenesis where increased AIM2 expression was seen around the necrotic core of atherosclerotic carotid lesions and in the vasa vasorum of neovasculature of aortic aneurysms (79). The NLRP3 inflammasome and AIM2 may thus have important roles in both *P. gingivalis*-induced periodontal disease and atherosclerosis through sustained inflammation.

Recently, the CD36/scavenger receptor (SR)-B2 was suggested to play a role at multiple points in *P. gingivalis*-mediated enhanced atherosclerosis in a mouse model (80). The study suggested that activation of the inflammasome by *P. gingivalis* is mediated by CD36/SR-B2 and

TLR2 which cause systemic release of pro-atherosclerotic IL-1 $\beta$  and macrophage pyroptosis. Systemic IL-1 $\beta$  activates vascular macrophages naïve to *P. gingivalis* to secrete IL-1 $\beta$  and promotes CD36-mediated uptake of oxLDL and increased formation of foam cells. The presence of oxLDL could inhibit *P. gingivalis*/*P. gingivalis* LPS-inflammasome activation and pyroptosis, which would allow greater atherosclerotic plaque to develop. TLR-CD36-/SR-B2-mediated IL-1 $\beta$  generation may thus be important to increase atherosclerotic lesions. Of note is also that cytoplasmic LPS sensing in human cells activates the non-canonical caspase-4-dependent inflammasome. This is a new mechanism of inflammasome activation where direct LPS-binding results in caspase oligomerization and activation leading to induction of IL-1 $\beta$  secretion and pyroptosis (81).

#### Alzheimer's disease

*P. gingivalis* may be an important pathogen in Alzheimer's disease (AD) contributing to brain inflammation (82). NLRP3 has been reported in microglial cells that responded to infection and initiation of neuro-degeneration in an Alzheimer's disease model (83). Furthermore, TLR2 and NLRP3 were recently found to co-operate to recognize a functional bacterial amyloid, curli fibers, in the plaques of brains in AD patients (84). Heneka et al. (85) found a strongly activated caspase-1 expression in human mild cognitive impairment and AD brains which suggested a role for the inflammasome in brain degenerative disease. AD brain deposits activated the NLRP3 inflammasome in microglial cells *in vitro* and *in vivo* which could lead to progression of AD (85–88). This suggested a role for the inflammasome in this neurodegenerative disease.

#### Non-alcoholic steatohepatitis

*P. gingivalis* seems to be a critical risk factor for progression of non-alcoholic steatohepatitis (NASH) through upregulation of the *P. gingivalis*-LPS-TLR2-pathway and by stimulating inflammasomes (89). These authors found that *P. gingivalis* exacerbated steatohepatitis induced by diet *via* induction of inflammasomes and inflammatory cytokines in the liver of mice. *P. gingivalis* was also demonstrated for the first time in the liver of NASH patients. It was suggested that dental infection with *P. gingivalis* promotes progression of NASH.

#### Squamous cell carcinoma

A close association has been found between *P. gingivalis* and squamous cell carcinoma (90). The fact that *P. gingivalis* modulates ATP/P2X<sub>7</sub>-signaling; secretes the anti-apoptotic enzyme, NDK, during infection of primary OECs; and expresses other virulence factors, such as fimbriae, gingipains, and PAD, that may be potential etiologic links to orodigestive cancers and other chronic diseases (58, 59). Of note is also that IL-1 $\beta$  promotes

malignant transformation of tumor aggressiveness in oral cancer (91).

### Rheumatoid arthritis

Periodontitis is more prevalent in patients with rheumatoid arthritis (RA) than in those without (92). RA is also prevalent in patients with periodontitis (93). Besides, there is improvement in RA after periodontal treatment and in periodontitis after treatment of RA (94, 95). Furthermore, DNA from a variety of oral bacteria, including *P. gingivalis*, has been detected in synovial fluid of active RA (96). Bostanci et al. (97) found a positive correlation between NLRP3 and expression of IL-1 $\beta$  and IL-18 in periodontitis, and upregulated levels of NLRP3, AIM2, and caspase-1 have been detected in gingival tissues of patients with periodontitis (14). Probably, *P. gingivalis* manipulates the host inflammatory responses to be able to survive and prevail within infected periodontal tissues (97), which it may achieve by limiting or controlling the activation of the NLRP3 inflammasome. It seems reasonable to expect similar effects in distant sites, for example, in the joints of rheumatic patients where *P. gingivalis* can be present.

### Concluding remarks

Inflammasomes represent a relatively new concept in innate immunity. There is great variation in inflammasomes and the mechanisms by which they detect and resist pathogens. Many interactions between inflammasomes and the innate immune system are still unknown. It is becoming clear that the inflammasome and its constituents are likely crucial in the initiation of periodontal disease and several chronic systemic diseases associated with periodontitis. Nevertheless, it may be difficult to intervene in inflammasome actions for the purpose of treating disease since interfering with key parts in the complex may have serious local and systemic effects. The ubiquitous distribution and importance of inflammasome activation in many peripheral processes adds to this limitation. There are still not fully understood roles of all players in the inflammasome complex where anti-inflammatory therapies might not be sufficient to treat the roots of the disease. Knowledge about inflammasomes has mainly been retrieved from murine systems. The applicability of some of these results to human cells is unclear because gene products differ between species, and the specificity of ligands is not always conserved. *P. gingivalis* has a number of ways for suppressing innate immunity and inflammasome activity. Although this subversion probably is important for periodontitis and some related systemic diseases, it remains to see if other parts of the oral microbiota can behave in a similar way and if this subversion can affect players other than *F. nucleatum* in the dental biofilm. Efforts should also be made to see how

inflammasomes can affect the ecology of the dental plaque microbiota.

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